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Steady-state phase or cooperative transitions between biochemical cycles

(enzyme lattice/cooperativity/free energy transduction/multicycle diagram)

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ABSTRACT **In a steady-state lattice of interacting enzyme molecules that have a multicycle kinetic diagram, a cooperative or phase transition may involve not only the conventional sudden change in the relative importance of the different states of a molecule but also a sudden change in the dominant cycles of the diagram. The latter effect implies a sudden switch in the dominant biochemistry (e.g., a sudden onset of active transport). An explicit example is discussed.**

This is the tenth paper in a series on interaction or cooperative effects between enzyme molecules at steady state. Most attention so far has been devoted to lattices of enzyme molecules with two-state cycles. From the point of view of statistical physics, this work amounts to an extension of the well-known two-state equilibrium Ising problem to steady states arbitrarily far from equilibrium. Currently we are examining systems with three-state cycles (with L. Stein; to be published) and with four states and three cycles. In both cases interesting and novel features are encountered. The latter work is the subject of the present paper.

Phase transitions in Ising and related steady-state systems (1–4) conventionally involve a sudden switch from the dominance of one of two discrete states to the other. But some steady-state enzyme-lattice systems may also exhibit a higher-order type of transition, not possible at equilibrium. This may occur if the kinetic diagram of the individual enzyme molecules of the lattice contains more than one cycle. In such cases the phase transition may bring about not only a sudden change in the dominant state or states but also a *sudden change in the dominant cycle or cycles*. Thus the predominant steady-state biochemistry taking place in the lattice may switch precipitously as a result of the phase transition. In the numerical example below, there is active transport of a ligand L (driven by ATP, say) after a phase transition but not before.

The simplest diagram that has more than one cycle and that allows coupling between two different thermodynamic forces is a diagram with four states and three cycles, as shown in Fig. 1*A*. We use this diagram here because our very limited purpose in this paper is simply to *illustrate*, with one hypothetical example, the general phenomenon described in the preceding paragraph. It remains to be seen, in the future, whether any natural or artificial steady-state enzyme-lattice systems exhibit this type of behavior.

Our discussion above has been in terms of a sharp phase transition. Of course a cooperative but nonprecipitous transition between dominant cycles would also be of considerable interest;

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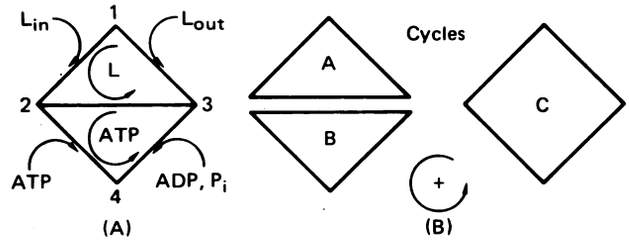

FIG. 1. (*A*) Four-state kinetic diagram for possible active transport of ligand L by ATP. (*B*) The three cycles that belong to this diagram. See text.

bacteriorhodopsin is a possible candidate.† A gradual transition could occur either in a lattice with subcritical interaction free energies (see below) or in a small aggregate or complex (e.g., of from two to six subunits). In the latter (finite) case, sharp behavior would not be observed no matter how strong the interactions.

The model and analytical properties for $\beta = 0$

Fig. 1*A* shows the four-state kinetic diagram we use (5, 6). There are three cycles (Fig. 1*B*); we take the counterclockwise direction as positive in all cycles. Without specifying the details of a biochemical mechanism, let us suppose for concreteness that in the positive direction in each case: cycle A transports a ligand L across a membrane from inside to outside; cycle B hydrolyzes ATP; and cycle C does both of these. Our primary interest is in cases where the cycle flux J_A and thermodynamic force X_A (for L) are both negative, J_B and X_B (for ATP) are both positive, and J_C and $X_A + X_B$ (the net force in cycle C) are also both positive. Thus, the "downhill" direction for transport of L is out → in (negative direction), but the ATP force X_B is large enough to overcome the negative X_A in cycle C so that L is transported "uphill" (in → out) in cycle C (5, 6).

The operational (total) flux in L is $J_1 = J_A + J_C$, while the operational flux in ATP is $J_2 = J_B + J_C$ (5, 6). If J_1 is positive, there is net flux of L in → out, against its electrochemical potential gradient (i.e., there is active transport of L by ATP).

We use the Bragg–Williams (or mean field) approximation to take care of nearest-neighbor interactions in the lattice (7). The rate constants chosen are shown in Fig. 2. Here $\alpha < 1$, β is very small, x is proportional to the ATP concentration (which can be varied), and y is an interaction parameter. Unity is used as a reference rate constant. To keep the mathematics as simple as possible, we assume one kind of interaction only: w_{44}, the free energy of interaction between neighboring molecules both in

† Hess, B., Korenstein, R. & Kuschmitz, D. (1978) *Sixth International Biophysics Congress, Kyoto, Japan*, 177 (abstr.).

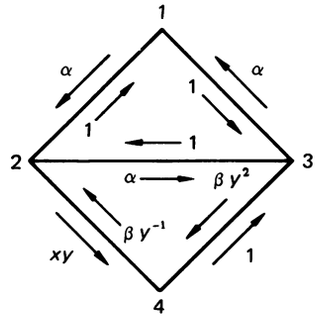

FIG. 2. Rate constants of the diagram, used in the example. See text.

state 4, is taken as zero or negative (relative to all other pair interactions). We define

$$y \equiv s^{p_4},\ s \equiv \exp[(z/2)(-w_{44}/kT)] \geq 1, \qquad [1]$$

where p_4 is the probability that any molecule in the lattice is in state 4, z is the nearest-neighbor number of the lattice, k is the Boltzmann constant, and T is absolute temperature. The factors y, y^{-1}, and y^2 in Fig. 2 then follow from the rather arbitrary choices $f_{24} = \frac{1}{2}$ and $f_{43} = 0$ in equation 40 of ref. 7. This model has the virtue of simplicity but the efficiency of free energy transduction $(-J_1X_1/J_2X_2)$ is small. More realistic models would include other interactions besides those of type 44.

Using the diagram method (5) and Fig. 2, it is easy to write each of the four steady-state p_i as a sum of eight terms divided by Σ, where Σ is the sum of all 32 terms. Also, the cycle fluxes and forces (5) are

$$J_A = (\alpha^3 - 1)(1 + \beta y^{-1})/\Sigma$$
$$J_B = (xy - \alpha\beta^2 y)(1 + \alpha)/\Sigma$$
$$J_C = (xy\alpha^2 - \beta^2 y)/\Sigma \qquad [2]$$
$$e^{X_1/kT} = \alpha^3,\ e^{X_2/kT} = x/\alpha\beta^2. \qquad [3]$$

The forces are independent of s. Because x and α are of order unity (see below) and $e^{X_2/kT}$ (ATP) is of order 10^{10}, β is of order 10^{-5}. In our explicit derivation of the p_i and the fluxes, below, we therefore take $\beta = 0$ for simplicity. (Incidentally, in the analysis below, the factor y^2 in the rate constant βy^2 in Fig. 2 is $e^4 = 54.6$ at the critical point.)

With $\beta = 0$, we find (5)

$$p_1 = (a + xy\alpha)/\Sigma,\ p_2 = a/\Sigma$$
$$p_3 = [a + xy(1 + \alpha)]/\Sigma,\ p_4 = axy/\Sigma$$
$$\Sigma = 3a + xyb$$
$$a \equiv 1 + \alpha + \alpha^2,\ b \equiv (1 + \alpha)(2 + \alpha),\ y \equiv s^{p_4} \qquad [4]$$

and

$$J_A = (\alpha^3 - 1)/\Sigma,\ J_B = xy(1 + \alpha)/\Sigma,\ J_C = xy\alpha^2/\Sigma$$
$$J_1 = J_A + J_C,\ J_2 = J_B + J_C. \qquad [5]$$

Note that $J_2 = p_4$. The ps and Js are regarded here as functions of x (or ATP concentration), for specified s and α.

In numerical work, we use

$$x = 3ap_4/(a - bp_4)y, \qquad [6]$$

which follows from the equation for p_4 above, to obtain $x(p_4)$ (rather than vice versa). The other quantities in Eqs. 4 and 5 are then calculated at each x value.

There are helpful symmetry properties that are not difficult to prove. First, we note from Eq. 6 that xy is a function of p_4 only (for a given α); call this function $F(p_4)$. If we then replace xy wherever it occurs in Eqs. 4 and 5 by $F(p_4)$, the Js and the other ps are all functions of p_4 that are independent of the value of s. Next, from Eq. 6, we see that the maximum value of $p_4(x \to \infty)$ is a/b. The half-maximum is $p_4^* \equiv a/2b$. One can then show (details omitted) that

$$F(p_4^* + \Delta)\,F(p_4^* - \Delta) = F(p_4^*)^2$$
$$x(p_4^* + \Delta)\,x(p_4^* - \Delta) = x(p_4^*)^2$$
$$P(p_4^* + \Delta) + P(p_4^* - \Delta) = 2P(p_4^*), \qquad [7]$$

where P represents here any one of the Js or ps.

There is a phase transition for large enough $-w_{44}/kT$. The critical properties follow from

$$\partial \ln x/\partial p_4 = 0,\ \partial^2 \ln x/\partial p_4^2 = 0 \qquad [8]$$

and Eqs. 7. We find

$$p_4^{(c)} = p_4^* = a/2b,\ \ln s_c = 4b/a,\ \Sigma_c = 6a$$
$$y_c = e^2,\ x_c = (3a/b)e^{-2},\ x_c y_c = 3a/b$$
$$p_1^{(c)} = (1/6) + (\alpha/2b),\ p_2^{(c)} = 1/6$$
$$p_3^{(c)} = (1/6) + [(1 + \alpha)/2b]$$
$$J_A^{(c)} = (\alpha^3 - 1)/6a,\ J_B^{(c)} = (1 + \alpha)/2b$$
$$J_C^{(c)} = \alpha^2/2b,\ J_2^{(c)} = a/2b$$
$$J_1^{(c)} = (\alpha^3 + 5\alpha^2 - \alpha - 2)/6b. \qquad [9]$$

The limiting values of the ps and Js as $x \to 0$ and $x \to \infty$ are easy to deduce from Eqs. 4 and 5; also these two limits are related by symmetry. For example,

$$J_1 \to [(\alpha - 1)/3] + [(2 - \alpha)x/9] + \ldots \quad (x \to 0)$$
$$J_2 = p_4 \to x/3 + \ldots \quad (x \to 0)$$
$$J_1 \to \alpha^2/b,\ J_2 = p_4 \to a/b \quad (x \to \infty). \qquad [10]$$

These limits are independent of s.

Numerical example (with $\beta = 0$)

In this example, we choose α so that $J_1^{(c)} = 0$. This value of α is 0.68740. The critical value of s is then $s_c = 4438.0$. If we take $z = 6$ (two-dimensional hexagonal lattice) in Eq. 1, the critical value of $-w_{44}/kT$ is 2.80. Fig. 3 shows the four steady-state ps as functions of x for $s = s_c$. That is, these are all critical curves. The critical value of x is $x_c = 0.19338$. Also, $p_4^{(c)} = p_4^* = 0.23815$. Before the transition, p_4 is small because x is small. But state 4 has the largest probability just after the transition because of the attractive 44 interactions. After the transition, p_2 is small because the rate constant xy out of state 2 (Fig. 2) is relatively large.

Fig. 4 (solid curves) shows the five critical ($s = s_c$) flux curves (recall that $J_2 = p_4$). With the choice $J_1^{(c)} = 0$ (see above), the flux J_1 (transport of L) is negative ("downhill", out → in) before

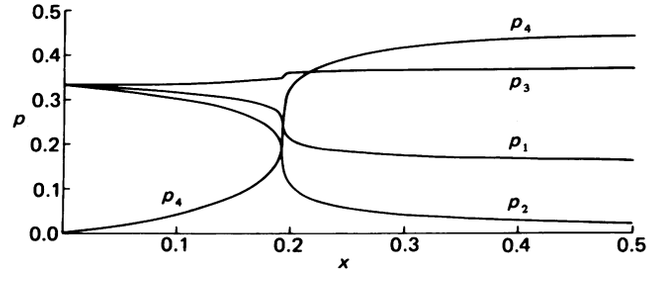

FIG. 3. The four steady-state state probabilities as functions of x, in a numerical example. See text.

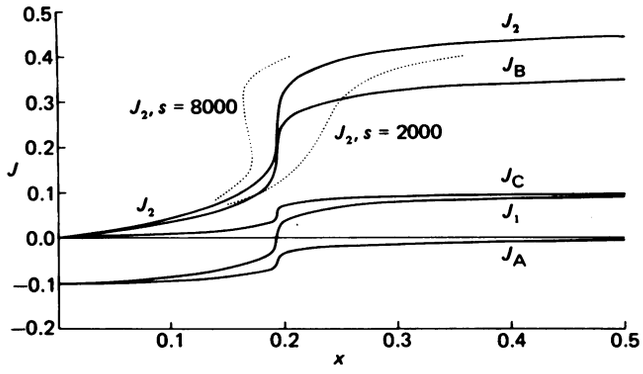

FIG. 4. The five critical flux curves, in a numerical example (s_c = 4438). Dotted curves give J_2 (= p_4) for s = 2000 and 8000. See text.

the transition but is positive ("uphill") after the transition. That is, as the ATP concentration is increased, the onset of active transport of L by ATP is sudden; the active transport exists after the transition but not before.

Cycle A is the dominant cycle at small x, while cycles B and C dominate at large x. This is true even in the absence of interactions (s = 1), but the change in cycle status occurs at relatively small values of x and is cooperative if s is large. Thus the midpoint of the transition (e.g., $J_2 = p_4 = p_4^*$ and $J_1 = 0$) occurs at x = 0.168 for s = 8000, at x = 0.193 for $s = s_c$ = 4438, at x = 0.234 for s = 2000, and at x = 1.429 for s = 1. Incidentally, these are all values of $x(p_4^*)$ (which depends on s).

The dotted J_2 (= p_4) curves in Fig. 4 illustrate the effect of varying s. Exactly analogous curves obtain for the other ps and Js. The transition is subcritical for s = 2000 (or for any $s < s_c$). A loop is found in the s = 8000 case (or for any $s > s_c$). Because of the symmetry of this model (Eqs. 7), the vertical cut (stable path) across the loop occurs at $x(p_4^*)$ = 0.168. [In fact, all of the curves in Figs. 3 and 4 would exhibit symmetry about the appropriate $x(p_4^*)$ value if we used ln x rather than x for the abscissa.]

In summary, in this steady-state model with a multicycle diagram, the phase transition involves not only a sudden change in the relative importance of the different states (Fig. 3) but also a sudden change in the relative importance of the different cycles (Fig. 4). The latter effect implies, in turn, a sudden switch in the dominant biochemistry (here, the onset of active transport).

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